U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-291 003

Effect of Thermal Stress and Total Residual Chlorination on Early Life Stages of the Mummichog 'Fundulus heteroclitus'

Environmental Research Lab., Gulf Breeze, FL

9 Dec 77

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/J-78-070	JOURNAL ARTICLE	PB291003

4. TITLE AND SUBTITLE	5. REPORT DATE
EFFECT OF THERMAL STRESS AND TOTAL RESIDUAL CHLORINATION ON EARLY LIFE STAGES OF THE MUMMICHOG FUNDULUS HETEROCLITUS	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
D. P. Middaugh, J. M. Dean, R. G. Domey, and G. Floyd	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
SAME AS BOX 12	1EA615
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory, Gulf Breeze Office of Research and Development U. S. Environmental Protection Agency Gulf Breeze, FL 32561	Final
	14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES
REFERENCE: "MARINE BIOLOGY" INTERNATIONAL JOURNAL ON LIFE IN OCEANS AND COASTAL WATERS, Springer-Verlag 1978, Germany. Vol. 46, No. 1, 1978, pp.1-8.

16. ABSTRACT
Effects of simultaneous short-term (7.5 to 60 min) thermal stress (24° to 34°C) and total residual chlorination (0.05 to 1.0 mg l^{-1}) on specific development stages of the mummichog Fundulus heteroclitus (Pisces: Cyprinodontidae), were investigated. For the embryonic stages, the total number of successfully hatched larvae was used as the criterion to measure effect. For the larval stages, survival 24 h after exposure was used. In the embryonic stages, temperature was the most important main variable. Only one embryonic stage (gastrula) was confounded by second-order interactions (temperature x duration of exposure x total residual chlorination). Both 0-day and 7-day-old larval stages showed significant higher-order interactions for all combinations of test parameters, suggesting the presence of synergistic effects of the three main experimental variables.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Marine fish Thermal stress	Residual chlorination Mummichog, Fundulus heteroclitus embryonic development larval mortality	08/A 06/F 06/C 06/T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Unlimited	Unclassified	10
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	PC A02/MF A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-78-070
JOURNAL ARTICLE

PB 291003

Offprint from "Marine Biology"
International Journal on Life in Oceans and Coastal Waters, Vol. 46, No. 1, March 1978, Pages 1–8

by Springer-Verlag 1978 · Printed in Germany

Effect of Thermal Stress and Total Residual Chlorination on Early Life Stages of the Mummichog Fundulus heteroclitus*

D. P. Middaugh[1], J. M. Dean[2], R. G. Domey[3] and G. Floyd[1]

1 Gulf Breeze Environmental Research Laboratory, Bears Bluff Field Station; John's Island, South Carolina, USA,

2 Belle W. Baruch Institute for Marine Biology and Coastal Research and Department of Biology, University of South Carolina; Columbia, South Carolina, USA and

3 Department of Bioengineering, University of Texas Health Center at San Antonio; San Antonio, Texas, USA

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Marine Biology 46, 1-8 (1978)

MARINE BIOLOGY
by Springer-Verlag 1978

1

Effect of Thermal Stress and Total Residual Chlorination on Early Life Stages of the Mummichog *Fundulus heteroclitus**

D.P. Middaugh[1], J.M. Dean[2], R.G. Domey[3] and G. Floyd[1]

[1]Gulf Breeze Environmental Research Laboratory, Bears Bluff Field Station; John's Island, South Carolina, USA,
[2]Belle W. Baruch Institute for Marine Biology and Coastal Research and Department of Biology, University of South Carolina; Columbia, South Carolina, USA and
[3]Department of Bioengineering, University of Texas Health Center at San Antonio; San Antonio, Texas, USA

Abstract

Effects of simultaneous short-term (7.5 to 60 min) thermal stress (24° to 34°C) and total residual chlorination (0.05 to 1.0 mg l^{-1}) on specific development stages of the mummichog *Fundulus heteroclitus* (Pisces: Cyprinodontidae), were investigated. For the embryonic stages, the total number of successfully hatched larvae was used as the criterion to measure effect. For the larval stages, survival 24 h after exposure was used. In the embryonic stages, temperature was the most important main variable. Only one embryonic stage (gastrula) was confounded by second-order interactions (temperature x duration of exposure x total residual chlorination). Both 0-day and 7-day-old larval stages showed significant higher-order interactions for all combinations of test parameters, suggesting the presence of synergistic effects of the three main experimental variables.

Introduction

Several coastal electric generating plants currently use estuarine water for single-pass cooling purposes and chlorinate the intake water to prevent biological fouling of condensor units (Lewis, 1966; Sorge, 1969). Estuarine organisms entrained in electric generating plant cooling systems are often damaged or destroyed by the combined effects of thermal stress and chlorination (Waugh, 1964; Morgan and Stross, 1969; McLean, 1973; Muchmore and Epel, 1973; Hoss *et al.*, 1975). Extensive estuarine or coastal thermal discharge plumes can affect organisms that have not been entrained but encounter elevated environmental temperatures singly and in combination with total residual chlorination (Markowski, 1960; Gameson *et al.*, 1961; Cory and Nauman, 1970; Stober and Hanson, 1974). Alderson (1972) found that larval plaice (*Pleuronectes platessa*) were more sensitive to free chlorination than developing embryos. Hoss *et al.* (1975) found that larval or juvenile flounder (*Paralichthys* sp.), menhaden (*Brevoortia tyrannus*), and mullet (*Mugil cephalus*) showed increased sensitivity to free chlorination when exposed at a temperature above their acclimation temperature.

Our study was designed to assess the effect of simultaneous thermal stress and exposure to total residual chlorination on specific developmental stages of an estuarine teleost, the mummichog *Fundulus heteroclitus*, which is one of the most common species of fish inhabiting the estuaries and *Spartina* spp. marshes of the eastern United States (Cain and Dean, 1976). *F. heteroclitus* is euryhaline and eurythermal, and found from the Gulf of St. Lawrence to Mexico (Bigelow and Schroeder, 1953). Because of its habitat requirements, the mummichog is likely to be affected by thermal or chemical additions to the estuarine environment.

Materials and Methods

Adult Spawning Stock

Adult *Fundulus heteroclitus* used as spawning stock were collected from We Creek, a tidal tributary of the North Edisto River estuary, South Carolina (Latitude 32° 38' 45" N; Longitude 80° 15' 25" W) in June and July, 1975. Ambient water

*Contribution No. 308 to the Gulf Breeze Environmental Research Laboratory. Contribution No. 183 to the Belle W. Baruch Institute for Marine Biology and Coastal Research.

0025-3162/78/0046/0001/$01.60

temperatures during collection and laboratory holding ranged from 26° to 31°C and salinity from 18 to 26‰. Twenty individuals of each sex were isolated in acrylic plastic flow-through aquaria (volume 18 l) in the laboratory. Each aquarium received 40 l h^{-1} of unfiltered seawater. Adults were fed the grass shrimp *Palaemonetes pugio* each day under fluorescent "cool white" lamps with 1400 lux of illumination (photoperiod, 14 h light:10 h dark).

Intramuscular injections of 50 International Units of Squibb Follutein[1] chorionic gonadotropin per fish were given anteriorly to the anal fins for 3 consecutive days. Forty-eight hours after the third (final) hormone injection, ripe eggs and milt were stripped into 200 mm diameter glass culture dishes. Each dish contained 200 ml of filtered 20‰ S seawater at 24°C ± 1 C°. After 5 min the fertilized demersal eggs were pipetted into hatchery jars. A Dyna-Flor® filter system was used to circulate water through each hatchery jar and a 40 l glass aquarium. A salinity of 20‰ and water temperature of 24°C ± 1 C°. were maintained in each hatchery jar and aquarium. Developing mummichog embryos were held in hatchery jars and the hatched larvae in the 40 l aquaria at the developmental salinity and temperature. Larvae used immediately after hatching (0-day-old) were not fed. Larvae used in tests 7 days after hatching were fed *Artemia* sp. nauplii each day from hatching until 24 h prior to use.

Thermal-Stress/Chlorination Exposures

The 6 specific developmental stages of the mummichog used and the corresponding stages (in parentheses) defined by Armstrong and Child (1965) were: 1-2 cell (Stages 2-3), gastrula (Stages 17-18), onset of circulation (Stage 25), 10-day embryo (Stage 35), 0-day-old larvae (Stage 37) and 7-day-old larvae (no corresponding stage).

Exposures to thermal stress and total residual chlorination (TRC) were conducted in 4 l acrylic plastic flow-through-treatment aquaria which received 18 l h^{-1} of temperature-controlled seawater. The exposure temperatures for each of the developmental stages at each TRC concentration were 24° (ambient), 29° and 34°C. Four nominal concentrations of chlorine (Mallinckrodt NaOCl, analytical reagent, minimum 5% NaOCl) were injected into 4 of the flow-through-treatment aquaria with Sage® Model 355 syringe pumps. An additional untreated aquarium was used for exposure of developmental stages to thermal stress alone. Measured TRC concentrations in respective treatment aquaria were 0.00 (control), and approximately 0.05, 0.1, 0.5 and 1.0 mg l^{-1}. Each developmental stage was exposed to each combination of thermal stress-TRC concentration for a duration of 7.5, 15, 30 and 60 min.

Exposure Procedures

Acrylic plastic exposure cups (7.5 cm inner diameter x 8.0 cm deep) with 300 µm nylon plankton netting bottoms were used for exposures. Thirty defined-stage embryos or larvae were placed in each of 2 exposure cups to provide treatment replication. Four sets of replicate exposure cups were then immersed in their respective treatment aquaria. A fifth set of cups was placed in the control aquarium (thermal stress only). Self-starting siphons in each treatment aquarium insured adequate water exchange in the exposure cups. Ambient salinities ranged from 18 to 24‰ during treatments. The dissolved oxygen level was monitored periodically with a YSI Model 51A oxygen meter. Dissolved oxygen was >6.0 mg l^{-1} during pre-treatment holding, treatment and post-treatment recovery intervals. The pH ranged from 7.5 to 7.8 during exposures.

After treatment, developing embryos were pipetted from their respective exposure cups, rinsed with clean seawater, and placed in 100 mm diameter glass culture dishes containing 60 ml of filtered seawater of 20‰ S at 24°C ± 1 C°. Seawater in the culture dishes was changed every 4 days, and observations of the total number of hatching larvae were made 16 days after the time of fertilization. Larvae exposed to each treatment level remained in their respective exposure cups throughout the test. Each set of replicate cups was removed from the treatment aquaria, rinsed with clean seawater, and resuspended in a 40 l glass aquarium containing aerated filtered seawater of 20‰ salinity at 24°C ± 1 C°. The number of survivors were counted 24 h after removal of exposure cups from the treatment aquaria.

Chemical Analysis

Total residual chlorination (TRC) concentrations (free + combined chlorine and other residual oxidants) were measured

[1]Mention of trade names does not imply endorsement by the U.S. Environmental Protection Agency.

Table 1. *Fundulus heteroclitus*. Nominal amounts of NaOCl added and measured concentrations of total residual chlorination (TRC) for tests conducted at 3 temperatures. SD: Standard deviation

Nominal NaOCl concentration (mg l^{-1})	Measured TRC concentrations (mg l^{-1})											
	24°C exposures				29°C exposures				34°C exposures			
	N	Range	$\bar{x}$	SD	*N*	Range	$\bar{x}$	SD	*N*	Range	$\bar{x}$	SD
5.6	12	0.77-1.29	0.99	0.16	12	0.84-1.20	1.06	0.12	12	0.82-1.30	1.27	0.53
3.2	12	0.37-0.57	0.46	0.06	12	0.36-0.86	0.53	0.15	12	0.40-0.57	0.50	0.05
1.8	12	0.07-0.13	0.10	0.02	12	0.08-0.15	0.10	0.02	12	0.06-0.12	0.10	0.02
1.0	12	0.04-0.08	0.07	0.02	12	0.02-0.08	0.06	0.02	12	0.04-0.07	0.06	0.01
0.0	12	0.00-0.00	0.00	0	12	0.00-0.00	0.00	0	12	0.00-0.00	0.00	0

with a Wallace and Tiernan amperometer titrator. Phenylarsine oxide and pH buffers used were standard Wallace and Tiernan reagents. The 5% KI solution and chlorine (Mallinckrodt NaOCl analytical reagent, minimum 5.0% NaOCl) stock solutions were prepared in deionized-distilled water. Stock solutions were stored in brown glass bottles at 4°C prior to use. Measurement of TRC concentrations employed procedures outlined in the standard methods of the American Public Health Association (1971). All water samples were taken directly from the treatment aquaria immediately prior to and just after conducting a bioassay. Agitation of samples was avoided to reduce the potential for error by photodecomposition or flashing-off of TRC (Environmental Protection Agency, 1974). Nominal amounts of NaOCl (mg l^{-1}) and measured ranges and means of TRC (mg l^{-1}) detected in each series of bioassays are shown in Table 1.

Statistical Analysis

The number of survivors in each container for each stage of development and treatment regime was counted and percent survival calculated. These data were converted to continuous data form by angular transformation, the arcsine transformation for proportions (Steele and Torrie, 1960). The transformed data were treated by a 4-way analysis of variance with replications (ANOVA) and the Student-Newman-Keul's (SNK) procedure (Snedecor, 1956; Sokal and Rholf, 1969). Each stage was analyzed separately. The ANOVA *F*-ratios showed the presence or absence of significant effects, and the SNK procedure indicated where significant differences occurred by comparisons among appropriate means. A systematic increase occurred in the hatch frequency in the control groups of the older developmental stages, probably due to the ease with which nonviable organisms could be identified and removed from the older stage samples (gastrula, onset of circulation and 10-day embryo) prior to testing. Because this bias precluded among-stages comparisons, the stages were analyzed separately.

Results

The significant *F*-ratios for all analyses of variance representing the 6 developmental stages of *Fundulus heteroclitus* are summarized in Table 2. The findings

Table 2. *Fundulus heteroclitus*. Locations of significant *F*-ratios for all stages of development, and corresponding P values. A: Temperature; B: duration of exposure; C: TRC; AB, AC, BC, ABC: interactions; R: replicates; NS: not significant

Development stage	Variables							
	A	B	C	AB	AC	BC	ABC	R
1-2 cell	≤.0001	NS	NS	NS	NS	NS	NS	NS
Gastrula	≤.01	NS	NS	NS	NS	NS	≤.01	NS
Circulation	≤.001	NS	NS	NS	NS	NS	NS	NS
10-day embryo	≤.0001	NS	NS	NS	NS	NS	NS	NS
0-day larvae	≤.001	≤.05	≤.001	≤.01	≤.001	≤.001	≤.001	NS
7-day larvae	≤.0001	≤.0001	≤.0001	≤.05	≤.0001	≤.01	≤.01	NS

Table 3. *Fundulus heteroclitus*, 1-2 cell stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2. obs: observations

ANOVA; grand mean = 49.30

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	2445.82	22.11	<0.0001
B	3	154.18	NS	
AB	6	94.81	NS	
C	4	76.71	NS	
AC	8	130.12	NS	
BC	12	169.30	NS	
ABC	24	53.76	NS	
R	1	110.32	NS	
Error term	60	110.64	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	43.07	46.75	58.07
N block obs	40.00	40.00	40.00

58.07 - 43.07 = 15.00 > 5.65 P <0.05
58.07 - 46.75 = 11.32 > 4.71 P <0.05

Table 4. *Fundulus heteroclitus*, gastrula stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2

ANOVA; grand mean = 40.88

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	450.73	6.87	<0.01
B	3	165.43	NS	
AB	6	94.73	NS	
C	4	77.53	NS	
AC	8	49.80	NS	
BC	12	161.50	NS	
ABC	24	145.75	2.46	<0.01
R	1	45.68	NS	
Error term	60	65.65	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	44.71	38.45	39.48
N block obs	40.00	40.00	40.00

44.71 - 38.45 = 6.26 > 4.34 P <0.05
44.71 - 39.48 = 5.23 > 3.62 P <0.05

Table 5. *Fundulus heteroclitus*, onset of circulation stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2

ANOVA; grand mean = 61.71

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	4535.35	44.75	<0.001
B	3	71.48	NS	
AB	6	82.83	NS	
C	4	48.94	NS	
AC	8	70.38	NS	
BC	12	103.88	NS	
ABC	24	102.68	NS	
R	1	0.10	NS	
Error term	60	101.35	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	74.00	55.20	55.93
N block obs	40.00	40.00	40.00

74.00 - 55.20 = 18.80 > 5.41 P <0.05
74.00 - 55.93 = 18.07 > 4.50 P <0.05

Table 6. *Fundulus heteroclitus*, 10-day embryo stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2

ANOVA; grand mean = 69.54

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	4966.03	46.54	<0.0001
B	3	51.27	NS	
AB	6	219.96	NS	
C	4	186.06	NS	
AC	8	57.52	NS	
BC	12	71.51	NS	
ABC	24	96.60	NS	
R	1	38.95	NS	
Error term	60	106.71	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	75.76	76.01	56.59
N block obs	40.00	40.00	40.00

76.01 - 56.59 = 19.42 > 5.55 P < 0.05
75.76 - 56.59 = 19.17 > 4.62 P < 0.05

Table 7. *Fundulus heteroclitus*, 0-day-old larval stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2

ANOVA; grand mean = 70.65

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	2799.12	61.03	<0.001
B	3	2539.19	55.36	<0.05
AB	6	334.81	7.30	<0.01
C	4	7685.87	167.58	<0.001
AC	8	1155.03	25.19	<0.001
BC	12	1035.77	18.88	<0.001
ABC	24	340.70	7.24	<0.001
R	1	165.27	NS	
Error term	60	45.86	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	79.16	70.35	62.44
N block obs	40.00	40.00	40.00

79.16 - 62.44 = 16.72 > 3.63 P <0.05
79.16 - 70.35 = 8.81 > 3.03 P <0.05
70.35 - 62.44 = 7.91 > 3.03 P <0.05

	Duration of exposure time (min)			
	7.5 min	15 min	30 min	60 min
Mean	78.97	76.83	67.95	58.85
N block obs	30.00	30.00	30.00	30.00

78.97 - 58.85 = 20.12 > 4.62 P <0.05
78.97 - 67.95 = 11.02 > 4.20 P <0.05
76.83 - 58.85 = 17.98 > 4.02 P <0.05
76.83 - 67.95 = 8.88 > 3.50 P <0.05
67.95 - 58.85 = 9.10 > 3.50 P <0.05

	Treatments: TRC concentration (mg l^{-1})				
	Control	1.0 mg	0.5 mg	0.1 mg	0.05 mg
Mean	81.97	41.93	64.15	82.60	82.60
N block obs	24.00	24.00	24.00	24.00	24.00

82.60 - 41.93 = 40.67 > 5.50 P <0.05
82.60 - 64.15 = 18.45 > 5.17 P <0.05
81.97 - 41.93 = 40.04 > 4.70 P <0.05
81.97 - 64.15 = 17.82 > 3.91 P <0.05
64.15 - 41.93 = 22.22 > 3.91 P <0.05

Table 8. *Fundulus heteroclitus*, 7-day-old larval stage. Locations of significant differences between means in the ANOVA as determined by the SNK test. For definition of variables see legend to Table 2

ANOVA; grand mean = 60.86

Source of variation	Degrees of freedom	Mean squares	*F*-ratio	P
A	2	1785.93	31.28	<0.0001
B	3	1675.32	29.17	<0.0001
AB	6	132.51	2.32	<0.05
C	4	16862.48	294.64	<0.0001
AC	8	563.39	9.84	<0.001
BC	12	673.66	11.77	<0.0001
ABC	24	315.06	5.51	<0.01
R	1	6.24	NS	
Error term	60	57.23	NS	

Student-Newman-Keuls test; P = 0.05

	Temperature (°C)		
	24°	29°	34°
Mean	67.86	60.18	54.55
N block obs	40.00	40.00	40.00

67.86 - 54.55 = 13.31 > 4.07 P <0.05
67.86 - 60.18 = 7.68 > 3.38 P <0.05
60.18 - 54.44 = 5.63 > 3.38 P <0.05

	Duration of exposure time (min)			
	7.5 min	15 min	30 min	60 min
Mean	68.81	65.18	57.02	52.44
N block obs	30.00	30.00	30.00	30.00

68.81 - 52.44 = 16.37 > 5.16 P <0.05
68.81 - 57.02 = 11.79 > 4.70 P <0.05
65.19 - 52.44 = 12.75 > 4.70 P <0.05
65.19 - 57.02 = 8.17 > 3.19 P <0.05
57.02 - 52.44 = 4.58 > 3.91 P <0.05

	Treatments: TRC concentration (mg l^{-1})				
	Control	1.0 mg	0.5 mg	0.1 mg	0.05 mg
Mean	77.81	14.29	64.44	74.85	772.94
N block obs	24.00	24.00	24.00	24.00	24.00

77.81 - 14.29 = 63.52 > 6.14 P <0.05
77.81 - 64.44 = 13.37 > 5.77 P <0.05
74.85 - 14.29 = 60.56 > 5.25 P <0.05
72.94 - 14.29 = 58.65 > 5.25 P <0.05
72.94 - 64.44 = 8.50 > 4.37 P <0.05
64.44 - 14.29 = 50.15 > 4.37 P <0.05

were: (1) all main variables and interactions had significant effects for the two larval stages tested (0-day and 7-day-old larvae); (2) the second-order interaction was significant for the gastrula stage; (3) no interactions were present for 3 of the 6 stages (1-2 cell, onset of circulation and 10-day embryos); (4) the *F*-ratio for temperature was significant for all stages. There were three stages (1-2 cell, onset of circulation and 10-day old embryos) where one of the main variables, temperature, was not confounded by any higher-order interactions which suggests that temperature variation may have accounted for the differential death rate in these stages. The remaining 3 stages (gastrula, 0-day and 7-day-old larvae) were characterized by significant second-order interactions in which temperature, TRC concentration and duration of exposure combined to produce a detrimental effect. At no stage of development were the *F*-ratios for replicates significant; this was interpreted as an indication of reliability of the experiment.

All significant differences for all main variables are presented to allow comparisons which locate the most likely sources of differences, although the exact degree of influence of the main variables cannot be assessed. The stages of development most vulnerable to stress by all variables were the 0-day and 7-day-old larvae, although all stages tested appeared to suffer ill effects, depending upon temperature. TRC concen-

trations and duration of exposure, within the ranges studied, were not effective as independent stresses. This observation should not be construed to mean that these two variables were neutral agents, for they were unequivocally implicated in the higher-order interactions which probably reflected the presence of synergistic effects.

The locations of significant differences between means in the analyses of variance for each developmental stage as determined by the SNK test are summarized in Tables 3-8. For the 1-2 cell and gastrula stages, overall effect was inversely related to temperature, the lower the temperature the greater the loss (Tables 3 and 4). For the onset of circulation and 10-day old embryo stages, the effect was reversed, with higher temperatures resulting in greater loss (Tables 5 and 6). In both the 0-day and 7-day-old larval stages (Tables 7 and 8) there was decreased survival with higher temperatures, increased TRC concentration and increased duration of exposure.

Discussion

Developing embryonic stages of *Fundulus heteroclitus* were more resistant to the deleterious effect of the experimental variables than 0-day and 7-day-old larvae in this study. For the embryonic stages, temperature was the only statistically significant main variable. TRC concentration and duration of exposure had no significant effect.

The eggs of many Cyprinodontidae, including the mummichog *Fundulus heteroclitus* and the marsh killifish *F. confluentus*, are spawned in rigorous environments where they may be exposed to rapid fluctuations in temperature and extended periods of desiccation (Harrington, 1959; Joseph and Saksena, 1966; Able and Castagna, 1975). This requires an egg with a thick chorion that effectively protects the embryo from external perturbations. The chorion may help to isolate developing mummichog embryos from the effect of TRC, but does not act as an effective shield against thermal stress.

We are unable to explain the inverse relationship between hatching success and test temperature for the 1-2 cell and gastrula stages, but two factors may have interacted to cause this response. The first is a lack of complete hardening of the chorionic membrane prior to experimental treatment, allowing penetration of TRC into the egg. The second factor involves the reaction kinetics and speciation of TRC products in seawater, which are not well understood. Dove (1970) stated that the ratio of oxidation products resulting from chlorination of seawater is temperature-dependent. In our toxicity tests with the 1-2 cell and gastrula stages, chlorination products with greater toxicity may have existed at the lower test temperature, thus resulting in greater mortality.

Exposure of developing mummichogs at the onset of circulation and 10-day embryo stages, in which the chorionic membrane had completed hardening (thus reducing the potential for TRC penetration), showed increased sensitivity with increased exposure temperatures. Hopkins and Dean (1975) also found that mummichog embryos were differentially sensitive to thermal stress as a function of developmental stage. They observed apparent changes in the mechanism of response according to the developmental stage at which embryos received the thermal shock. Although the exposure temperatures were lower in the present study, there is also evidence of thermally induced effect.

Experimental results for 0-day and 7-day-old larvae show that these stages are susceptible to each of the main variables (temperature, TRC concentration and duration of exposure) and to all interactions. In a study of the toxicity of chlorine to the plaice *Pleuronectes platessa*, Alderson (1972) found that developing embryos were less sensitive than the larvae. Alderson (1974) also found sensitivity to chlorine decreased with increasing larval age for *P. platessa*, and for the Dover sole *Solea solea*.

In this study, 7-day-old larvae appeared more sensitive to the combined effect of thermal stress and TRC than were 0-day-old larvae, especially for the exposures of 7.5, 15 and 30 min duration. Middaugh and Dean (1977) showed that 7-day-old *Fundulus heteroclitus* larvae were more sensitive to cadmium than 1-day-old larvae. Increased sensitivity in 7-day-old larvae was attributed to increased gill function (Armstrong and Child, 1965) with resultant gill-specific toxicant activity for cadmium (Gardner and Yevich, 1970). Middaugh *et al.* (1977a, b) demonstrated that the pseudobranch and gills were the structures most affected in marine fish larvae exposed to lethal concentrations of TRC. It is possible that a similar destructive mechanism resulted in toxicity of TRC to 0-day and 7-day-old mummichog larvae in the present study, with the toxicity accentuated by the general responses to increased temperature and duration of exposure.

Because of their reproductive strategy, it is unlikely that developing mummichog embryos would be subjected to en-

trainment in power station cooling systems. However, the developing embryos of pelagic spawners as well as larvae of the mummichog and other species could be entrained. This study has demonstrated the potentially detrimental effect of entrainment of eggs and larvae of an estuarine fish in coastal electric generating plants using chlorination to prevent biological fouling. While direct comparisons among development stages could not be made, results suggest that effective assessment of environmental perturbations may require the use of a number of life-history stages.

Acknowledgements. We thank Mr. P.W. Lempesis who helped us obtain the nearly 22,000 embryos and larvae used in this study. The participation of J.M. Dean was supported in part by a grant from the Energy Research and Development Administration E(38-1)-869.

Literature Cited

Able, K.W. and M. Castagna: Aspects of an undescribed reproductive behavior in *Fundulus heteroclitus* (Cyprinodontidae) from Virginia. Chesapeake Sci. *16*, 282-284 (1975)

Alderson, R.: Effects of low concentrations of free chlorine on eggs and larvae of plaice, *Pleuronectes platessa* L. *In:* Marine pollution and sea life, pp 312-315. Ed. by M. Ruivo. London: Fishing News (Books) 1972

- Seawater chlorination and the survival and growth of the early developmental stages of plaice, *Pleuronectes platessa* L., and Dover sole, *Solea solea* (L.). Aquaculture *4*, 41-53 (1974)

American Public Health Association: Standard methods for the examination of water and wastewater, 13th ed. 834 pp. New York, N.Y.: American Water Works Association & Water Pollution Control Federation 1971

Armstrong, P.B. and J.S. Child: Stages of normal development of *Fundulus heteroclitus*. Biol. Bull. mar. biol. Lab., Woods Hole *128*, 143-168 (1965)

Bigelow, H.B. and W.C. Schroeder: Fishes of the Gulf of Maine. Fishery Bull. Fish Wildl. Serv. U.S. *53*, 1-577 (1953)

Cain, R.L. and J.M. Dean: Annual occurrence, abundance and diversity of fish in a South Carolina intertidal creek. Mar. Biol. *36*, 369-379 (1976)

Cory, R.L. and J.W. Nauman: Epifauna and thermal additions in the Upper Patuxent River estuary. Chesapeake Sci. *10*, 210-217 (1970)

Dove, R.A.: Reaction of small dosages of chlorine in seawater, 189 pp. Leatherhead, Surrey, England: Electric Generating Board, South Eastern Region 1970 (Research Report No. 42/70 File No. O.3070/ID Job No. 10665)

Environmental Protection Agency: Handbook of analytical quality control in water and wastewater laboratories, 45 pp. Cincinatti, Ohio: Analytical Quality Control Laboratory, National Environmental Research Center 1974

Gameson, A.L.H., H. Hall and W.S. Preddy: Effects of heated discharges on the temperature of the Thames estuary. II. Combustion, N.Y. *32*, 37-45 (1961)

Gardner, G.R. and P.P. Yevich: Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Bd Can. *27*, 2185-2196 (1970)

Harrington, R.W.: Delayed hatching in stranded eggs of marsh killifish, *Fundulus confluentus*. Ecology *40*, 430-437 (1959)

Hopkins, S.R. and J.M. Dean: The response of developmental stages of *Fundulus* to acute thermal shock. *In:* Physiological ecology of estuarine organisms, pp 301-318. Ed. by F.J. Vernberg. Columbia: University of South Carolina Press 1975

Hoss, D.E., L.C. Coston, J.P. Baptist and D.W. Engle: Effects of temperature, copper and chlorine on fish during simulated entrainment in power plant condensor cooling systems, pp 519-527. Vienna: International Atomic Energy Agency 1975 (Ref. IAEA-SM-189/19)

Joseph, E.B. and V.P. Saksena: Determination of salinity tolerances in mummichog, *Fundulus heteroclitus*, larvae obtained from hormone-induced spawning. Chesapeake Sci. *7*, 193-197 (1966)

Lewis, B.G.: Chlorination and mussel control. I: The chemistry of chlorinated seawater, a review of the literature, 10 pp. Leatherhead, Surrey, England: Central Electricity Research Laboratory 1966 (Lab. Note cent. Elect. Res. Lab. No. RD/LN106/66, Job No. 10266)

Markowski, S.: Observation on the response of some benthonic organisms to power station cooling water. J. Anim. Ecol. *29*, 349-357 (1960)

McLean, R.I.: Chlorine and temperature stress in estuarine invertebrates. J. Wat. Pollut. Control Fed. *45*, 837-841 (1973)

Middaugh, D.P., J.A. Couch and A.M. Crane: Response of early life history stages of the striped bass, *Morone saxatilis* to chlorination. Chesapeake Sci. *18*, 141-153 (1977a)

- A.M. Crane and J.A. Couch: Toxicity of chlorination to juvenile spot, *Leiostomus xanthurus*. Wat. Res. *11*, 1089-1096 (1977b)
- and J.M. Dean: Comparative sensitivity of eggs, larvae and adults of the estuarine teleosts, *Fundulus heteroclitus* and *Menidia menidia* to cadmium. Bull. envir. Contam. Toxicol. *17*, 645-652 (1977)

Morgan, R.P. and R.G. Stross: Destruction of phytoplankton in the cooling water supply of a steam electric station. Chesapeake Sci. *10*, 165-171 (1969)

Muchmore, D. and D. Epel: The effects of chlorination of wastewater on fertilization in some marine invertebrates. Mar. Biol. *19*, 93-95 (1973)

Sokal, R.R. and J.F. Rholf: Biometry, 776 pp. San Francisco: W.H. Freeman & Co. 1969

Sorge, E.V.: The status of thermal discharges east of the Mississippi River. Chesapeake Sci. *10*, 131-138 (1969)

Snedecor, G.W.: Statistical methods, 5th ed. 534 pp. Ames, Iowa: Iowa State University Press 1956

Steele, R.G.D. and J.H. Torrie: Principles and procedures in statistics, 481 pp. New York: McGraw-Hill 1960

Stober, Q.J. and C.H. Hanson: Toxicity of chlorine and heat to pink (*Oncorhynchus gorbusca*) and chinook salmon (*O. tshawytascha*). Trans. Am. Fish. Soc. *103*, 569-576 (1974)

Waugh, G.D.: Observations on the effects of chlorine on the larvae of oysters (*Ostrea edulus* L.) and barnacles (*Eliminius modestus* Darwin). Ann. appl. Biol. *54*, 423-440 (1964)

Douglas P. Middaugh
U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Bears Bluff Field Station
P.O. Box 368
John's Island, South Carolina 29455
USA

Date of final manuscript acceptance: December 9, 1977. Communicated by J.M. Lawrence, Tampa